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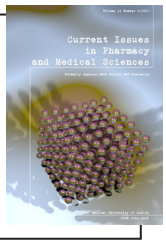

The study of phenolic compounds and antioxidant activity of raw materials of *Heliopsis helianthoides* (l.) sweet

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ABSTRACT

Using high performance liquid chromatography (HPLC), we studied the chemical composition and antioxidant activity of bioactive substances in the roots, leaves, flowers and seeds of *Heliopsis helianthoides*. The results of our study showed the presence of 5 phenolic compounds in its roots, 4 phenolic compounds in its leaves, 10 phenolic compounds in its flowers and 8 phenolic compounds in its seeds. The highest content of identified compounds was found in the leaves of this plant – 3192.20±79.78 mg/kg. The dominating hydroxycinnamic acid was chlorogenic acid. This had its highest concentration (1537.21±38.43 mg/kg) in the *Heliopsis helianthoides* leaves. Among flavonoids, luteolin prevailed in the roots, apigenin-7-glucoside prevailed in the seeds and rutin prevailed in the leaves and flowers. Maximum rutin content (1426.64±35.67 mg/kg) was found in the *Heliopsis helianthoides* leaves.

Antioxidant activity study *in vitro* uncovered the substantial antioxidant potential of bioactive substances (BASs) in all tested samples of the raw materials, being within the limits of 2.81-8.13 mg/g. Most active in this respect were *Heliopsis helianthoides* leaves. The obtained data indicate the feasibility of the development of new antioxidant active drugs on the basis of raw materials of *Heliopsis helianthoides.*

INTRODUCTION

The importance of antioxidants in human life is very high as they prevent the free radicals formation in cells which in turn may favor the appearance of various immune and contagious diseases, as well as provoke the development of cancer, diabetes, arthritis, atherosclerosis, Alzheimer's disease, etc. [1,2]. Therefore, the search for new herbs possessing expressed antioxidant activity is an urgent aspect of contemporary pharmacy and medicine.

Heliopsis helianthoides (L.) Sweet (*Asteraceae* family) is a perennial herbaceous plant originating from Mexico and several Latin American countries. The plant is cultivated in many countries as an ornamental plant [3]. Any information relating to its chemical composition and medical applications is scarce as per today, but other species of *Heliopsis* Pers. are being studied, and the obtained results hint at good prospects in the research of this plant genus. For example, in Heliopsis longipes raw material, according to Aguilar *et al*. (2008) [4], analgesic activity was found, whereas, in the roots of *Heliopsis helianthoides* var. scabra Hajdu *et al*. (2014) [5,6], cerebral tumor metastasis inhibiting capacity was established. Moreover, *Heliopsis sinaloensis* is known to possess antioxidant and anti-mutagenic action [7].

The antioxidant activity is mostly connected to a high content of phenolic compounds as testified by numerous studies, in particular, for *Asteraceae* family plants. Islam *et al*. (2016) [8], as well as Karamać *et al*. (2012) [1], for example, studied *Helianthus annuus* seeds and demonstrated that high content of phenolic compounds favors the expressed antioxidant activity.

This work dwells on the study of phenolic compounds in the leaves, flowers, seeds and roots of *Heliopsis helianthoides*, as well as on establishing the antioxidant activity in these raw materials.

MATERIAL AND METHODS

We analyzed the dried and milled roots, flowers, leaves and seeds of *Heliopsis helianthoides*. Flowers and leaves were collected within the blossoming period in June-July, seeds and roots in September. The raw materials were collected in 2018-2019 in the territories of Kharkiv and

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Khmelnitskiy Regions, Ukraine. Drying of raw materials was carried out by utilizing the air-shadow method.

The qualitative composition and quantitative contents of phenolic compounds in tested raw materials were studied by means of HPLC.

For study of phenolic compounds in *Heliopsis helianthoides* raw materials, as well as determination of antioxidant activity via HPLC method, we used standard samples of substances and solvents for chromatographic analysis from Merck KGaA (Darmstadt, Germany).

Phenolic compounds were separated at a liquid chromatograph consisting of a quarter pump, online degasser, column temperature controller, SIL-30AC automatic sampler, CTO-20AC thermostat and a SPD-M20A DAD (diode matrix detector). In the course of study, Wise Clean WUC-A06H ultrasonic cleaning set and Class A analytical balance were used in the conformity with the State Pharmacopoeia of Ukraine 2.0.1.

HPLC identification of the phenolic compounds was performed by the comparison of retention times (tR) and UV spectra with standard substances analyzed under the same conditions.

Quantitative determination was undertaken at the wavelength of maximum absorption of known flavonoids and phenolics, in particular, hydroxycinnamic acids: luteolin – 350 nm; 6,7-dihydroisoflavone – 244 nm; rutin – 353 nm; formononetin – 248 nm; 7-oxyisoflavone – 242 nm; apigenin-7-glucoside – 340 nm; hyperoside and isoquercitrin – 350 nm; gallic acid – 270 nm; neochlorogenic, chlorogenic, caffeic and rosmarinic acids – 320 nm.

Extraction

Materials for analysis were prepared by extracting 0.3 g milled raw materials with 10 ml methanol within 20 min using an ultrasonic bath at 20±2°C. Prior to usage, the obtained extracts were passed through an 0.45 μm membrane filter [9]. The extraction method was optimized for the studied raw materials.

Phenolic compounds

The chromatographic separation of the phenolic compounds was performed by means of 250 mm × 4.6 mm ACE C18 columns with granularity at 5.0 μm (Pennsylvania, USA). Elution flow rate was 1 ml/min. The mobile phase binary solvent system consisted of solvent A (0.1% formic acid aqueous solution) and solvent B (acetonitrile). All solvents passed through ultrasonic degassing and 0.23 μm pore size membrane filtering. The linear mobile phase gradient was as follows (Table 1).

Table 1. Linear mobile phase gradient

Time, min	0.1% formic acid aqueous solution in acetonitrile, %
0-8	5-15
8-30	15-20
30-48	20-40
48-58	40-50
58-65	50
65-66	50-95

The column had constant temperature of 25°C, 10 μl samples were injected [3]

The total amount of phenolic compounds (as pyrogallol) in *Heliopsis helianthoides* raw materials was also determined by spectrophotometry according to the State Pharmacopoeia of Ukraine method. This method is harmonized with the monograph of the European Pharmacopoeia.

Antioxidant potential

The antioxidant potential of *Heliopsis helianthoides* raw materials was studied by way of applying the HPLC method, using a Waters 2695 chromatograph (Waters Corporation, Milford, CT, USA) with Waters 996 PDA photodiode matrix detector (Waters Corporation).

Extracts of *Heliopsis helianthoides* raw materials were obtained in the same way as for the study of the phenolic compounds.

Chromatographic separation was performed using an ACE C18 column of 250 mm long, 4.6 mm diameter and granularity of 5.0 μm (Pennsylvania, USA). Elution flow rate was 1 ml/min. The mobile phase solvent system consisted of solvent A (0.1% trifluoroacetic acid aqueous solution) and solvent B (acetonitrile). All solvents passed through ultrasonic degassing and 0.45 μm pore size membrane filtering [10,11]. The linear mobile phase gradient was as follows (Table 2).

Table 2. Linear mobile phase gradient

Time, min	0.1% trifluoroacetic acid aqueous solution in acetonitrile, %
0-8	5-15
8-30	15-20
30-48	20-40
48-58	40-50
58-65	50
65-66	50-95

The column had constant temperature of 25°C. 10 μl samples were injected [10,11]

After passing through the HPLC-PDA detector system, the mobile phase containing the tested samples was fed via Gilson 305 pump (Middleton, WI, USA) to the column via a mixing tee with ABTS reagent in split relation of 1:1. A Teflon column (Waters PCR module, Milford, CT, USA) 3 m long and 0.25 mm in diameter was used, its granularity being 1.58 μm. ABTS solution system control parameters included: column temperature circa 50°C, mobile phase flow rate 0.5 μl [10,11].

Sample color change in mixture with ABTS reagent after reaction ending was recorded using a Waters 2487 UV/VIS detector (Waters Corporation) at wavelength of 650 nm [10,11].

In selecting the analysis terms, we were guided by signal value expressed in negative peak height as a sensitivity indicator. Antioxidant potential of tested samples was determined by the comparison with that of Trolox standard solution in eight different concentrations within the range of 0.625-80 mg/ml. The constructed calibration plot was expressed with the following quadratic equation:

$$-1.54 \cdot 10^2 x^2 + 4.16 \cdot 4.16 \cdot 10^4 x - 2.08 \cdot 10^4;$$
$$R^2 \text{ (ABTS)} = 0.9991.$$

Antioxidant potential of extracts (X, mg/g) was calculated by formula:

$$X = \frac{m_0 \cdot 20000}{m_1 \cdot (100 - w)}$$

where m_0 – mass of Trolox standard sample, g; m_1 – mass of tested sample, g; w – moisture content in raw material, % [10,11].

RESULTS

As the result of our study, we identified 5 phenolic compounds in the *Heliopsis helianthoides* roots, 4 phenolic compounds in its leaves, 10 phenolic compounds in its flowers and 8 phenolic compounds in its seeds. In all tested types of raw materials, we confirmed the presence of neochlorogenic and chlorogenic acids. Caffeic acid, however, was not found in seeds, nor was rutin found in the roots of the plant. Moreover, phenolic gallic acid was identified only in the flowers and seeds of *Heliopsis helianthoides*, while the flavonoids luteolin and 6,7-dihydroisoflavon were identified in its roots and seeds, and 7-oxyisoflavon was identified in the flowers and seeds of the plant. Hydroxycinnamic rosmarinic acid and flavonoids hyperoside, isoquercetrin and formononetin were found in flowers; apigenine-7-glucoside was found in the seeds of *Heliopsis helianthoides*.

HPLC chromatograms of phenolic compounds in *Heliopsis helianthoides* roots are presented in Figure 1, in leaves in Figure 2, in flowers and seeds in Figure 3 and Figure 4, respectively. Qualitative composition and quantitative contents (as dry matter) of phenolic compounds in *Heliopsis helianthoides* raw materials are specified in Table 3.

The highest total content of identified phenolic compounds was within *Heliopsis helianthoides* leaves at 3192.20 ±79.81 mg/kg. Total content of phenolic compounds in flowers (674.26±16.86 mg/kg) and seeds (698.75±17.47 mg/kg) of this plant was barely identical and was 4.7 and 4.6 times, respectively, less than in leaves. The lowest phenolics content was evident in the roots – 369.27±9.23 mg/kg.

Among the hydroxycinnamic acids, in all raw material samples, chlorogenic acid dominated. As regards the flavonoids, rutin prevailed in leaves and flowers, luteolin prevailed in seeds, apigenine-7-glucoside prevailed in roots.

Neochlorogenic acid was mostly accumulated in *Heliopsis helianthoides* flowers (48.54±1.21 mg/kg). Its content in roots was 1.7 times lower than in the flowers, in leaves – 3.6 times, in seeds – 6.6 times less. Chlorogenic (1537.21±10.12 mg/kg) and caffeic (214.78±5.37 mg/kg) acids highest content was recorded in the leaves of the plant. Its seeds contained chlorogenic acid 2.5 times less, and flowers and roots almost 4 and 5 times, respectively, less than the leaves. Rutin was mostly concentrated in *Heliopsis helianthoides* leaves (1426.64±35.67 mg/kg).

Heliopsis helianthoides roots accumulated more hydroxycinnamic acids (362.91±9.07 mg/kg), 85% of which was chlorogenic acid (310.80±7.77 mg/kg). Flavonoids content in this part of plant was only 6.36±0.16 mg/kg (less than 2% of the total content of identified compounds). In addition, luteolin content in the plant roots (5.16±0.13 mg/kg) was 4.3 times less than that of 6,7-dihydroisoflavone (1.20±0.03 mg/kg), while the content of hydroxycinnamic acids (1765.56±44.14 mg/kg) and flavonoid rutin in *Heliopsis helianthoides* leaves was almost identical. Chlorogenic acid accounted for circa 87% of the sum of hydroxycinnamic acids.

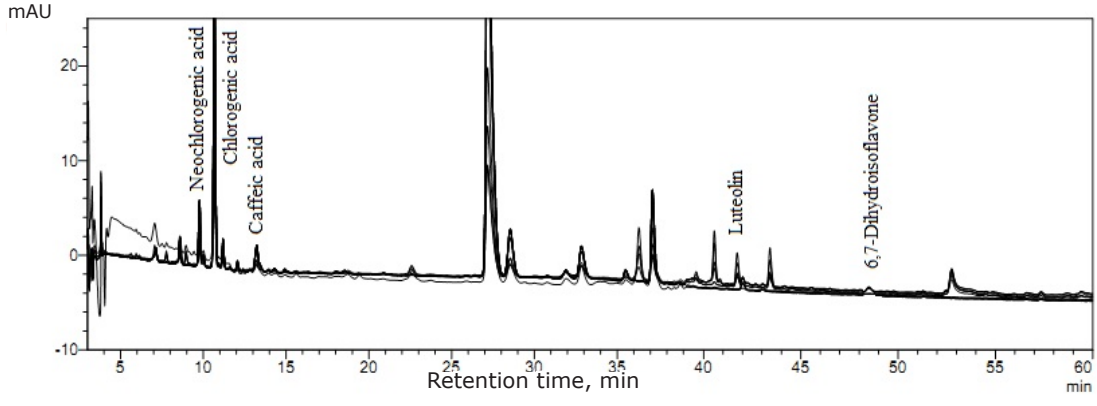

Figure 1. HPLC chromatogram of phenolic compounds in roots of *Heliopsis helianthoides*

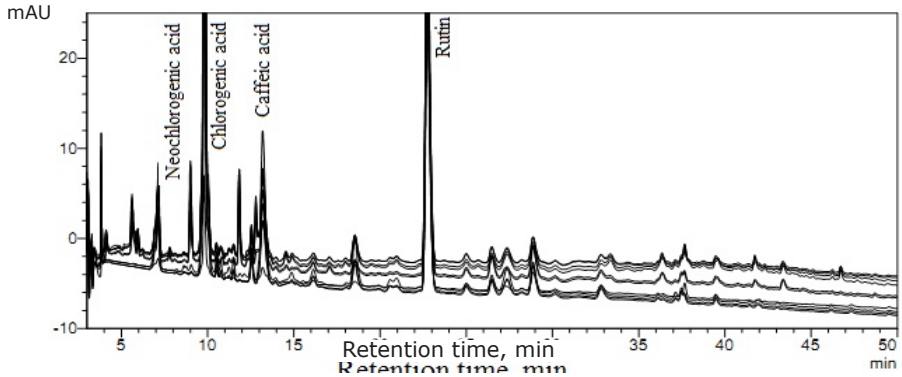

Figure 2. HPLC chromatogram of phenolic compounds in leaves of *Heliopsis helianthoides*

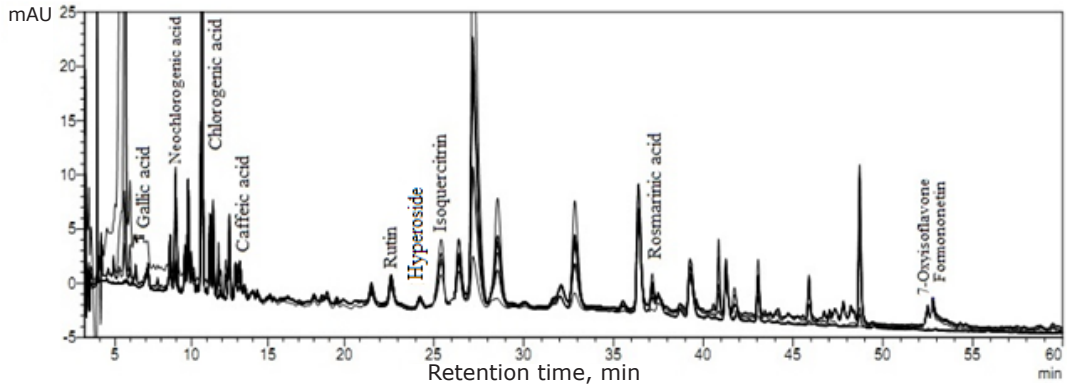

Figure 3. HPLC chromatogram of phenolic compounds in flowers of *Heliopsis helianthoides*

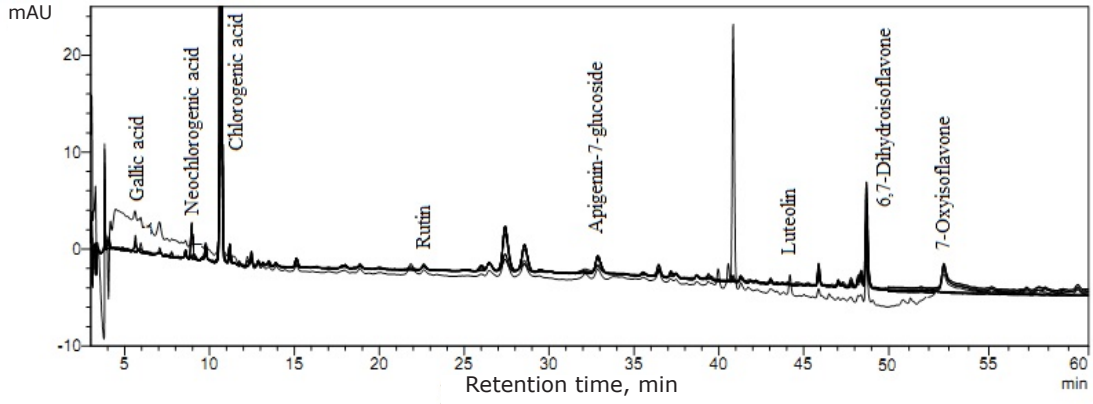

Figure 4. HPLC chromatogram of phenolic compounds in seeds of *Heliopsis helianthoides*

Table 3. Qualitative composition and quantitative contents (as dry matter) of phenolic compounds in *Heliopsis helianthoides* raw materials

Compound	Roots		Leaves		Flowers		Seeds	
	tR, min	Quantitative content, mg/kg	tR, min	Quantitative content, mg/kg	tR, min	Quantitative content, mg/kg	tR, min	Quantitative content, mg/kg
Phenolic acids								
Gallic acid	—	—	—	—	5.96	7.42±0.19	5.64	6.70±0.17
Total content of the phenolic acids	—	—	—	—	—	7.42±0.19	—	6.70±0.17
Hydroxycinnamic acids								
Neochlorogenic acid	8.60	27.17±0.68	9.06	13.57±0.34	8.61	48.54±1.21	8.60	7.48±0.18
Chlorogenic acid	10.68	310.80±7.77	9.81	1537.21±38.43	10.68	404.75±10.12	10.68	616.74±15.42
Caffeic acid	13.24	24.94±0.62	13.20	214.78±5.37	13.15	18.93±0.47	—	—
Rosmarinic acid	—	—	—	—	37.17	74.20±1.86	—	—
Total content of hydroxycinnamic acids	—	362.91±9.07	—	1765.56±44.14	—	546.42±13.66	—	624.22±15.60
Flavonoids								
Rutin	—	—	22.75	1426,64±35.67	22.63	74.78±1.87	22.62	19.58±0.49
Hyperoside	—	—	—	—	24.01	1.33±0.03	—	—
Isoquercetrin	—	—	—	—	24.91	22.66±0.57	—	—
Apigenin-7-glucoside	—	—	—	—	—	—	32.88	28.84±0.72
Luteolin	42.03	5.16±0.13	—	—	—	—	43.06	0.34±0.01
6,7-Dihydroisoflavone	47.93	1.20±0.03	—	—	—	—	47.79	0.41±0.01
Formononetin	—	—	—	—	52.76	16.20 ± 0.41	—	—
7-oxyisoflavone	—	—	—	—	52.75	5.45±0.14	52.76	18.66±0.47
Total content of flavonoids	—	6.36±0.16	—	1426.64±35.67	—	120.42±3.01	—	67.83±1.70
Total content of identified compounds	—	369.27±9.23	—	3192.20±79.81	—	674.26±16.86	—	698.75±17.47

Results are expressed as means ± SD of three measurements; $p<0.05$; «—» - not identified

The flowers of *Heliopsis helianthoides* contained 4.5 times more hydroxycinnamic acids (546.42±13.66 mg/kg) than the flavonoids (120.42±3.01 mg/kg). Chlorogenic acid accounted for circa 75% of the total content of hydroxycinnamic acids in this part of plant. Rosmarinic acid content was of 74.20±1.86 mg/kg, almost 5.5 times less than that of chlorogenic acid. *Heliopsis helianthoides* flowers contained 5 flavonoids of 120.42±3.01 mg/kg – that is 4.5 times less than the hydroxycinnamic acids content. Rutin (74.78±1.87 mg/kg) made 62% of the total flavonoids, whereas, isoquercetrin content (22,66±0.57 mg/kg) was 3 times less and formononetin was 4.6 times less than that of rutin. The content of hyperoside and 7-oxyisoflavon in *Heliopsis helianthoides* flowers was negligible: 1.33±0.03 and 5.45±0.14 mg/kg, respectively.

Heliopsis helianthoides seeds contained flavonoids 9 times less than the hydroxycinnamic acids. All 5 identified flavonoids made in sum 67.83±1.70 mg/kg. Among them, apigenin-7-glucoside prevailed. This possessed circa 42.5% of total flavonoids in this part of plant. The quantity of rutin and 7-oxyisoflavone was almost identical: 19.58±0.49 and 18.66±0.47 mg/kg, respectively, barely 1.5 times less than that of apigenin-7-glucoside. The contents of luteolin and 6,7-dihydroisoflavone was below 0.5 mg/kg.

The total amount of phenolic compounds (as pyrogallol) in *Heliopsis helianthoides* raw materials by spectrophotometry (as dry matter): in the roots was 1.61±0.07%, in the flowers – 3.27±0.11%, in the leaves – 4.82±0.19%, in the seeds – 3.59±0.14%.

The antioxidant activity of bioactive substances was studied using HPLC and was expressed as Trolox equivalent. The HPLC chromatogram is shown using the example of *Heliopsis helianthoides* leaves in Figure 5. The results of this experiment are presented in Table 4.

In the course of our study it was established that the highest antioxidant potential of BASs was found in *Heliopsis helianthoides* (L.) Sweet leaves: 8.13±0.23 mg/g. In flowers (4.89±0.11 mg/g), this parameter was 1.6 times lower. The antioxidant activity of all bioactive substances in roots and seeds of *Heliopsis helianthoides* was nearly equal: 3.24±0.08 and 2.81±0.07 mg/g, respectively.

Table 4. Antioxidant activity of BASs of *Heliopsis helianthoides* raw materials

Part of plant	Trolox equivalent antioxidant capacity, mg/g
Roots	3.24±0.08
Leaves	8.13±0.23
Flowers	4.89±0.11
Seeds	2.81±0.07

Results are expressed as means ± SD of three measurements; p<0.05

DISCUSSION

It is difficult to compare our results on phenolic compounds and antioxidant activity of *Heliopsis helianthoides* raw materials to the results of other researchers. This species of *Heliopsis* Pers. has not yet been studied in detail. Therefore, we are only able to compare our analytical results to those for other Heliopsis Pers. species. For example, Olivas-Quintero *et al*. (2017) [7] studied the phenolic compounds and pharmacological action of *Heliopsis sinaloensis* B. L. Turner.

By applying the HPLC method to *Heliopsis sinaloensis* leave methanol extract, these authors identified such flavonoids as cinnamic acid, two kaempferol 7-O-rhamnosylhexosides and rosmarinic acid, while on application to a stem methanol extract, rosmarinic acid and 3'-O-(8″-Z-caffeoyl) rosmarinic acid were indicated.

They also found that *Heliopsis sinaloensis* leaves possessed a higher content of flavonoids and phenolic acids as compared to stems. As regards the pharmacological activity, they noted the expressed antioxidant effect of *Heliopsis sinaloensis* raw material methanol extracts, and ascertained that antioxidant action was connected with the availability of, not only phenolic compounds, but of other substance classes as well. As regards the phenolic compounds, they specified the substantial contribution of rosmarinic acid to the development of the above-mentioned activity [7].

Wu (2007) [12] and Liang *et al*. (2016) [13] presented their conclusions on the high antioxidant activity of chlorogenic acid. Some researchers also studied, substantiated and confirmed the high antioxidant activity of rutin [14-16]. The results of our study lie in total correlation with the assertions expressed in the above works. Still, we may also conclude that a more detailed understanding of *Heliopsis helianthoides* raw materials antioxidant activity mechanism is necessary, particularly of components making decisive contribution to the development of this activity.

CONCLUSIONS

The obtained results showed that within the studied *Heliopsis helianthoides* raw materials, most phenolic compounds were accumulated in its leaves. The study of antioxidant activity indicated this activity was available in all raw material samples. The maximum antioxidant activity

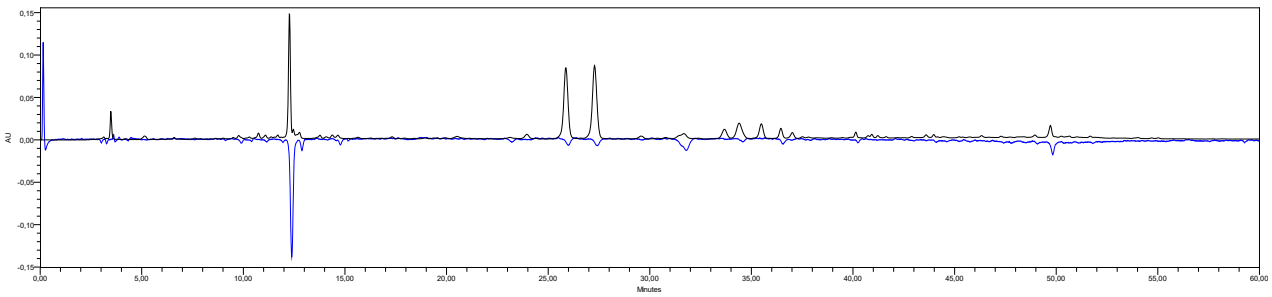

Figure 5. HPLC chromatogram determinantion of antioxidant activity of *Heliopsis helianthoides* leaves

was found in *Heliopsis helianthoides* leaves, and correlated with the high content of chlorogenic acid and rutin in this part of plant. The experimental results enabled deeper and more generalized knowledge as regards the quantitative composition and qualitative content of phenolic-origin compounds in *Heliopsis helianthoides* raw materials, as well as the biological action of bioactive substances contained therein. The obtained data will be used for standardizing *Heliopsis helianthoides* raw materials and the development of antioxidant drugs on its basis.

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